

GLOTZER et al. Appl. No. 09/881,736 Attorney Docket No. 0652.2260001/EKS/AES

Amendments to the Claims

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The listing of claims will replace all prior versions and listings of claims in the application.

1-44. (cancelled)

- 45. (currently amended) A method for determining whether a compound has the potential to inhibit cytokinesis by determining the compound's ability to inhibit the function of a CYK-4 protein comprising amino acid residues 360-536 of SEQ ID NO:2 or a fragment of the CYK-4 protein to promote GTP hydrolysis by a Rho family GTPase, the method comprising:
- (i) incubating the Rho family GTPase with GTP for a period of time sufficient to allow saturation of the Rho family GTPase's GTP binding sites;
- (ii) adding the a CYK-4 protein comprising amino acid residues 360-536 of SEQ ID NO:2 or the fragment of the CYK-4 protein to the Rho family GTPase and the GTP in the presence and absence of the compound, wherein the CYK-4 protein or the fragment of the CYK-4 protein, in the absence of the compound, stimulates GTP hydrolysis by the Rho family GTPase; and
- (iii) determining an amount of GTP that is hydrolyzed in the presence and absence of the compound;

wherein the compound is determined to have the potential to inhibit cytokinesis if the compound inhibits the CYK-4 stimulated GTP hydrolysis determined in (iii) ; and

wherein the CYK 4 protein is selected from the group consisting of human CYK 4 (SEQ ID NO:2), a polypeptide which stimulates GTP hydrolysis by the Rho family GTPase and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1, murine CYK 4 (SEQ ID NO:4), and a polypeptide which stimulates GTP hydrolysis by the Rho family GTPase and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:3, wherein the stringent conditions comprise (i) overnight incubation at 42 °C in a solution comprising 50% formamide, 5x SSC, wherein 1 x SSC comprises 150 mM NaCl and 15 mM trisodium citrate, 50 mM sodium phosphate, pH 7.6, 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by (ii) washing in 0.1x SSC at about 65 °C.

46. (previously presented) The method of claim 45, wherein the Rho family GTPase is a full-length Rho family GTPase protein or a fragment of the Rho family GTPase protein that retains GTPase activity.

47. (cancelled)

48. (currently amended) The method of claim 46, wherein the CYK-4 protein is selected from the group consisting of human CYK-4 (SEQ ID NO:2) and a polypeptide which stimulates GTP hydrolysis by the Rho family GTPase and which has an amino

acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1.

- 49. (previously presented) The method of claim 48, wherein the Rho family GTPase is selected from the group consisting of human RhoA, human RhoB, human RhoC, human RAC1, human RAC2, human RAC3, and human GB25.
- 50. (previously presented) The method of claim 49, wherein the Rho family GTPase is human RhoA.
- 51. (previously presented) The method of claim 46, wherein the Rho family GTPase is immobilized on a solid support.
- 52. (previously presented) The method of claim 46, wherein the GTP is labeled.
- 53. (previously presented) The method of claim 52, wherein the GTP is labeled with a radioisotope or a fluorescent label.
- 54. (currently amended) A method for determining whether a compound has the potential to inhibit cytokinesis by determining the compound's ability to inhibit the function of a CYK-4 protein comprising amino acid residues 1-120 of SEQ ID NO:2 or a fragment of the CYK-4 protein to bind to a member of the MKLP1 subfamily of kinesin-like proteins, the method comprising:

- (i) incubating the a CYK-4 protein comprising amino acid residues 1-120 of SEQ ID NO:2 or the fragment of the CYK-4 protein for a period of time with the MKLP1 protein subfamily member [[,]] in the presence and absence of the compound, wherein the CYK-4 protein or the fragment of the CYK-4 protein, in the absence of the compound, binds the MKLP1 protein subfamily member; and
- (ii) determining an amount of the MKLP1 protein subfamily member bound to the CYK-4 protein or the fragment of the CYK-4 protein in the presence and absence of the compound;

wherein the compound is determined to have the potential to inhibit cytokinesis if the compound inhibits the binding of the CYK-4 protein or the fragment of the CYK-4 protein to the MKLP1 protein subfamily member as determined in (ii) ; and

— wherein the CYK-4 protein is selected from the group consisting of human CYK-4 (SEQ ID NO:2), a polypeptide which binds the MKLP1 protein-subfamily member and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1, murine CYK-4 (SEQ ID NO:4), and a polypeptide which binds the MKLP1 protein-subfamily member and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:3, wherein the stringent conditions comprise (i) overnight incubation at 42 °C in a solution comprising 50% formamide, 5x SSC, wherein 1 x SSC comprises 150 mM NaCl and 15 mM trisodium citrate, 50 mM sodium phosphate, pH 7.6, 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml

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denatured, sheared salmon sperm DNA, followed by (ii) washing in 0.1x SSC at about 65 °C.

- 55. (currently amended) The method of claim 54, wherein the MKLP1 protein subfamily member is a full-length MKLP1 protein or a fragment of the MKLP1 protein subfamily member that binds the CYK-4 protein or the fragment of the CYK-4 protein.
- 56. (previously presented) The method of claim 55, wherein the MKLP 1 protein subfamily member is selected from the group consisting of CeM03D4.1b (SEQ ID NO:7) and HsMKLP1 (SEQ ID NO:8).
- 57. (previously presented) The method of claim 56, wherein the MKLP 1 protein subfamily member is HsMKLP1 (SEQ ID NO:8).
- 58. (cancelled)
- 59. (currently amended) The method of claim 56, wherein the CYK-4 protein is selected from the group consisting of human CYK-4 (SEQ ID NO:2) and a polypeptide which binds the MKLP1 protein subfamily member and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1.
- 60. (cancelled)

- 61. (currently amended) The method of claim 55, wherein the CYK-4 protein or the fragment of the CYK 4 protein is immobilized on a solid support, and wherein the MKLP1 protein subfamily member or the fragment of the MKLP1 protein subfamily member is labeled.
- 62. (previously presented) The method of claim 61, wherein the label is a radioisotope, a fluorescent label, or a hapten.
- The method of claim 55, wherein step (i) is performed in 63. (previously presented) solution.
- 64. (currently amended) A method for determining whether a compound has the potential to inhibit cytokinesis by determining the compound's ability to inhibit CYK-4 function by determining the compound's ability to inhibit self association of a CYK-4 protein comprising amino acid residues 1-120 of SEQ ID NO:2 or a fragment of the CYK-4 protein, the method comprising:
- (i) incubating in the presence and absence of the compound a first CYK-4 protein comprising amino acid residues 1-120 of SEQ ID NO:2 or a fragment of the first CYK-4-protein with a second CYK-4 protein comprising amino acid residues 1-120 of SEQ ID NO:2 or a fragment of the second CYK-4-protein, wherein, in the absence of the compound, the first CYK-4 protein or the fragment of the first CYK-4 protein binds to

the second CYK-4 protein or the fragment of the second CYK-4 protein, and wherein the second CYK-4 protein or the fragment of the second CYK-4 protein is labeled; and

(ii) determining an amount of the second CYK-4 protein or the fragment of the second CYK-4 protein bound to the first CYK-4 protein or the fragment of the first CYK-4 protein;

wherein the compound is determined to have the potential to inhibit cytokinesis if the compound inhibits the binding of the first CYK-4 protein or the fragment of the first CYK-4 protein to the second CYK-4 protein or the fragment of the second CYK-4 protein as determined in (ii); and

wherein the first CYK-4 protein and the second CYK-4 protein are each selected from the group consisting of human CYK-4 (SEQ ID NO:2), a polypeptide which binds to the first CYK-4 protein or the second CYK-4 protein and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1, murine CYK-4 (SEQ ID NO:4), and a polypeptide which binds to the first CYK-4 protein or the second CYK-4 protein and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:3, wherein the stringent conditions comprise (i) overnight incubation at 42 °C in a solution comprising 50% formamide, 5x SSC, wherein 1 x SSC comprises 150 mM NaCl and 15 mM trisodium citrate, 50 mM sodium phosphate, pH 7.6, 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by (ii) washing in 0.1x SSC at about 65 °C.

65-66. (cancelled)

The method of claim 64, wherein the first CYK-4 protein 67. (currently amended) is selected from the group consisting of human CYK-4 (SEQ ID NO:2) and a polypeptide which binds to the first CYK-4 protein or the second CYK-4 protein and which has an amino acid sequence encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide having a nucleotide sequence as set forth in SEQ ID NO:1.

68. (cancelled)

69. (cancelled)

70. (currently amended) The method of claim 64, wherein the first CYK-4 protein or the fragment of the first CYK-4 protein is immobilized on a solid support, and wherein the second CYK-4 protein or the fragment of the second CYK-4 protein is labeled.

The method of claim 70, wherein the second CYK-4 71. (currently amended) protein or the fragment of the second CYK-4 protein is labeled with a radioisotope label, a fluorescent label, a hapten label, a peptide label, or an enzyme label.

72. (currently amended) The method of claim 64, wherein the first CYK-4 protein or the fragment of the first CYK-4 protein is identical to the second CYK-4 protein or the fragment of the second CYK-4 protein.

73. (currently amended) The method of claim 64, wherein the first CYK-4 protein or the fragment of the first CYK-4 protein is different from the second CYK-4 protein or the fragment of the second CYK-4 protein.

74-77. (cancelled)